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Pesticide metabolism in plants and microorganisms

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Understanding pesticide metabolism in plants and microorganisms is necessary for pesticide development, for safe and efficient use, as well as for developing pesticide bioremediation strategies for contaminated soil and water. Pesticide biotransformation may occur via multistep processes known as metabolism or cometabolism. Cometabolism is the biotransformation of an organic compound that is not used as an energy source or as a constitutive element of the organism. Individual reactions of degradation–detoxification pathways include oxidation, reduction, hydrolysis, and conjugation. Metabolic pathway diversity depends on the chemical structure of the xenobiotic compound, the organism, environmental conditions, metabolic factors, and the regulating expression of these biochemical pathways. Knowledge of these enzymatic processes, especially concepts related to pesticide mechanism of action, resistance, selectivity, tolerance, and environmental fate, has advanced our understanding of pesticide science, and of plant and microbial biochemistry and physiology. There are some fundamental similarities and differences between plant and microbial pesticide metabolism. In this review, directed to researchers in weed science, we present concepts that were discussed at a symposium of the American Chemical Society (ACS) in 1999 and in the subsequent book *Pesticide Biotransformation in Plants and Microorganism: Similarities and Divergences,* edited by J. C. Hall, R. E. Hoagland, and R. M. Zablotowicz, and published by Oxford University Press, 2001.

Nomenclature: American Chemical Society; fenchlorazole-ethyl; glutathione; glutathione-*S*-transferase; naphthalic anhydride; polyaromatic hydrocarbons; polychlorinated biphenyls; reductive dehalogenation; trichloroethylene.

Key words: Biotransformation, degradation, enzyme, herbicide, metabolic fate, pesticide, xenobiotic.

Presently, there are ca. 900 pesticide products and ca. 600 active pesticidal ingredients on the market (Hall et al. 2001b). Millions of tons of pesticides are applied annually; however, less than 5% of these products are estimated to reach the target organism, with the remainder being deposited on the soil and nontarget organisms, as well as moving into the atmosphere and water (Pimental and Levitan 1986). The metabolic fate of pesticides is dependent on abiotic environmental conditions (temperature, moisture, soil pH, etc.), microbial community or plant species (or both), pesticide characteristics (hydrophilicity, $pK_{a/b}$, K_{ow}, etc.), and biological and chemical reactions. Abiotic degradation is due to chemical and physical transformations of the pesticide by processes such as photolysis, hydrolysis, oxidation, reduction, and rearrangements. Further, pesticides may be biologically unavailable because of compartmentalization, which occurs as a result of pesticide adsorption to soil and soil colloids without altering the chemical structure of the original molecule. However, enzymatic transformation, which is mainly the result of biotic processes mediated by plants and microorganisms, is by far the major route of detoxification.

Metabolism of pesticides may involve a three-phase process (Table 1) (Hatzios 1991; Shimabukuro 1985). In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar, amino acid, or glutathione, which increases the water solubility and reduces toxicity compared with the parent pesticide. Generally, Phase II metabolites have little or no phytotoxicity and may be stored in cellular organelles. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also nontoxic (Hatzios 1991). In leafy spurge (*Euphorbia esula* L.), examples of Phase III metabolism are the conjugation of the *N*-glycoside metabolite of picloram with malonate and the formation of a gentibioside from the picloram glucose ester metabolite (Frear et al. 1989) (Figure 1).

There are fundamental similarities and differences between plant and microbial pesticide metabolism. This review encompasses the enzymatic transformations of a wide variety of pesticides and presents the mechanism, biochemistry, genetics, and regulation of these processes in plants and microbes. Furthermore, this article focuses on the broad aspects of pesticide metabolism in plants and microorganisms and examines the importance of these biochemical pathways for pesticide development and environmental stewardship.

This review is a synopsis of a symposium that took place at the 218th national meeting of the American Chemical Society (ACS) in New Orleans, LA, in August 22 to 26, 1999. The Weed Science Society of America was one of the supporters and sponsors of this symposium. Subsequent to this symposium was a recent book, *Pesticide Biotransformation in Plants and Microorganisms: Similarities and Divergences,* ACS Symposium Series 777, published by Oxford University Press (Hall et al. 2001a). The book provides an

TABLE 1. Summary of the three phases of pesticide metabolism (adapted from Shimabukuro 1985).

Characteristics	Initial properties	Phase I	Phase II	Phase III
Reactions	Parent compound	Oxidation, hydrolysis, reduction	Conjugation	Secondary conjugation or incorporation into biopolymers
Solubility	Lipophilic	Amphophilic	Hydrophilic	Hydrophilic or insoluble
Phytotoxicity	Toxic	Modified or less toxic	Greatly reduced or nontoxic	Nontoxic
Mobility	Selective	Modified or reduced	Limited or immobile	Immobile
Bioavailability[a]	***	***	**	* Or unavailable

[a] ***, Readily absorbed in GI tract of animals; **, less absorption; *, limited absorption.

accumulation of some of the most recent research on enzymes from plant and microorganisms that catalyze pesticide metabolism. The purpose of the symposium and book on plant and microbial pesticide transformation was to bring together scientists from a variety of disciplines such as biochemistry, microbiology, plant physiology, and toxicology to present, summarize, and update information on xenobiotic metabolism. Specific enzymes and processes included hydrolytic enzymes, glutathione and other conjugation mechanisms, cytochrome P450 oxidases, peroxidases, nitroaromatic transformations, and reductive dehalogenation (RDE). The symposium also highlighted concepts of bioremediation, pesticide degradation in the rhizosphere, herbicide metabolism, crop safeners, and in vitro methods for studying pesticide biotransformation. It is hoped that this review summarizes the current knowledge of the metabolic action on pesticides in a manner that will be useful to students, researchers, instructors, and others involved in the discipline of weed science.

Primary Metabolism

Oxidative Transformations

Reactions by Cytochromes P450

Oxygenation is the most frequent first step in the biotransformation of pesticides and other organic xenobiotics. Many of these reactions are mediated by oxidative enzymes, e.g., cytochrome P450s, peroxidases, and polyphenol oxidases. The most extensively studied oxidative enzymes in plants and animals are the P450s, which are the most important enzymes in Phase I pesticide metabolism (Barrett 2000). Cytochrome P450s are hemethiolate proteins that have been characterized in animals, plants, bacteria, and filamentous fungi. In plants, bacteria, and fungi, P450s produce many secondary metabolites including plant growth regulators, isoprenoids, and alkaloids. Cytochrome P450s are encoded by a superfamily of genes designated as *CYP,* which have highly conserved residues around the heme portion of the protein (Barrett 2000). The first plant P450 gene

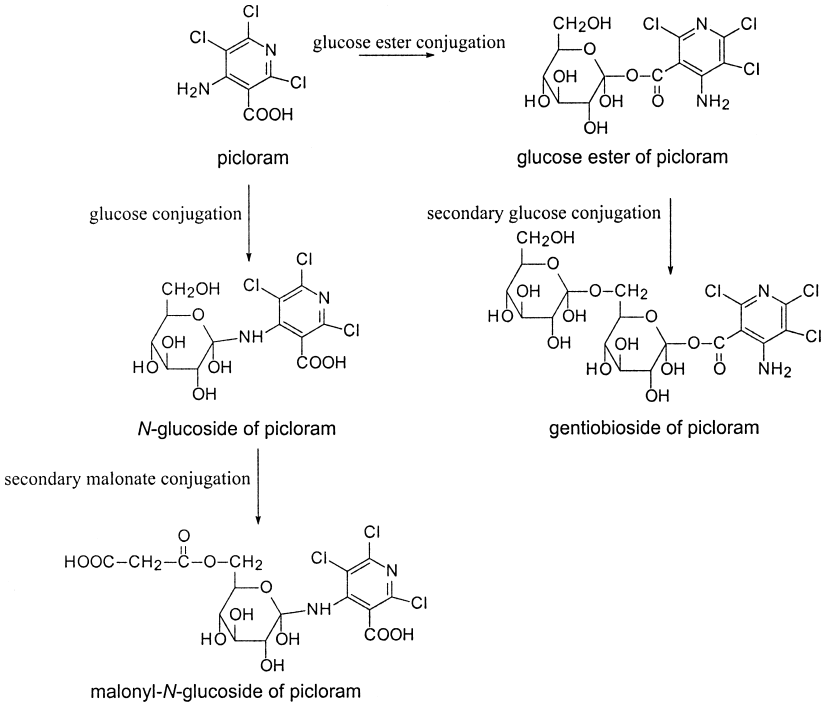


FIGURE 1. Conjugation and secondary conjugation of picloram in leafy spurge (*Euphorbia esula* L.) as proposed by Frear et al. (1989).

was sequenced in 1990 (Bolwell et al. 1994), and presently, more than 500 P450 plant genes have been described (Barret 2000). P450 genes occur in clusters in the genome (Frey et al. 1997). Regulation and expression of P450s are not well understood in plants or microorganisms mainly because of the very low quantities of P450 enzymes usually present in these cells, particularly if the organism has not been exposed to physiochemical, physiological, or xenobiotic stress.

Cytochrome P450s often catalyze monooxygenase reactions, usually resulting in hydroxylation, according to the following reaction: $RH + O_2 + NAD(P)H + H^+ \rightarrow ROH + H_2O + NAD(P)^+$. However, there are many other P450-mediated reactions including dehydration, dimerization, deamination, dehydrogenation, heteroatom dealkylation, epoxidation, reduction, and C–C or C=N cleavage. P450s are divided into three classes. Class I P450s are flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) dependent, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) requiring P450s that are usually microsomal membrane-bound proteins in plants and filamentous fungi. Bacteria and nonfilamentous fungi class I P450s are in soluble form (van den Brink et al. 1998). Class II P450s are similar to those in class I, but they are found only in bacterial and animal mitochondria. Class III P450s are located in plant plastids and do not require auxillary redox partners.

Agrochemicals can influence cytochrome P450 systems by acting as effectors, thereby modifying pesticide metabolism, or by modulating overall metabolism of an organism. These effects can increase or decrease physiological activities, which may affect growth and development. Pioneering work on P450-mediated herbicide metabolism in plants was conducted using the phenylurea herbicides, particularly chlortoluron. On the whole-plant level, wheat (*Triticum aestivum* L.) seedlings exposed to chlortoluron and known cytochrome P450 inhibitors (e.g., piperonyl butoxide or 1-aminobenzotriazole) were injured more than plants treated with chlortoluron alone (Cabanne et al. 1987; Gaillardon et al. 1985). Similar results were observed using plant cell suspension cultures, where a P450 inhibitor, tetcyclacis, reduced chlortoluron metabolism (Canivenc et al. 1989). Direct evidence that xenobiotic metabolism was mediated by P450s was obtained through experimentation with plant microsomal preparations. Using microsomal preparations from several plant species, it was shown that chlortoluron was metabolized to two metabolites by at least two different P450 enzymes (Mougin et al. 1990). Since that time, a number of P450-mediated phenylurea-metabolizing genes have been characterized (Robineau et al. 1998; Shiota et al. 1996; Siminszky et al. 1999).

Mougin et al. (2001) demonstrated that the fungicide fenpropimorph was metabolized to an oxygenated metabolite in wheat seedling microsomal preparations. Increased metabolism occurred when seeds were pretreated with naphthalic anhydride, a chemical safener that enhances cytochrome P450 levels. Further, oxidation of fenpropimorph in wheat seedling microsomes was inhibited when the preparations were exposed to carbon monoxide, which binds to the heme portion of the P450 molecule instead of oxygen, thereby blocking enzymatic reactions. These authors suggested that fenpropimorph metabolism is P450-mediated. Other researchers have used microsomes to demonstrate that the mechanism of resistance to several dissimilar herbicide chemistries in blackgrass (*Alopecurus myosuroides*) (Menendez and De Prado 1997) and rigid ryegrass (*Lolium rigidum*) (Preston et al. 1996) was based on enhanced P450-mediated metabolism. Herbicide resistance mediated by P450s may arise via two scenarios: (1) mutation of an existing P450, allowing increased binding and metabolism of the herbicide or (2) increased activity of existing P450s (Barrett 2000). In the future, researchers will no doubt continue to focus on isolating and characterizing plant P450 genes associated with pesticide metabolism. With a better understanding of P450 genes and their regulation, it may be possible to manipulate the crop plant system to increase herbicide tolerance.

Peroxidases, Phenoloxidases, and Related Oxidoreductases

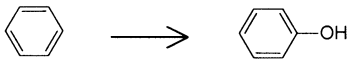
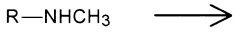
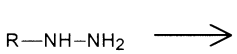
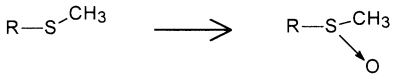
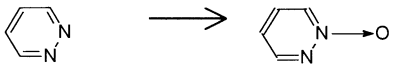
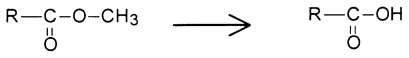
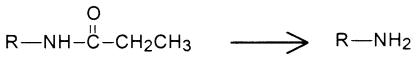
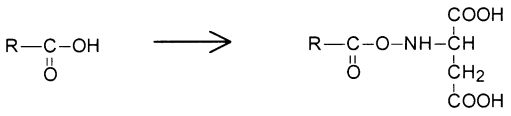
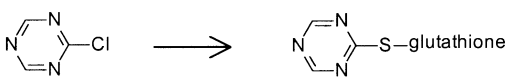
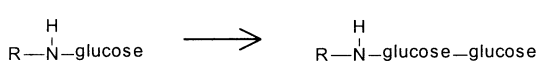
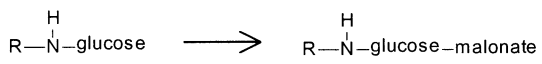
Plants and microorganisms produce a wide range of oxidative enzymes (e.g., peroxidase, polyphenoloxidase, laccase, and tyrosinase) other than P450s that catalyze the polymerization of various anilines and phenols (Dec and Bollag 2001). For example, peroxidase-mediated pesticide transformations in plants that function similar to P450s include decarboxylation, sulfur oxidation, *N*-demethylation, ring hydroxylation, and aromatic methyl group oxidations (Lamoureux and Frear 1979) (Table 2). In plants, peroxidase enzymes often function in Phase III metabolism, e.g., formation of bound residues. Horseradish (*Amorocia lapathifolia* Gilib.) roots contain large quantities of peroxidase. Horseradish root tissue has been used to remove 2,4-dichlorophenol from water and was more effective in contaminant removal than the purified peroxidase enzyme (Dec and Bollag 2001).

White rot fungi (*Phanerochaete chrysosporium*) offer high potential for xenobiotic transformation because they possess free radical–based lignin degrading systems (lignin peroxidase and manganese-dependent peroxidases) that can degrade a wide range of pollutants such as polychlorinated biphenyls (PCBs) and nitroaromatic explosives (Barr and Augst 1994). In most instances, polymerization products have reduced toxicity compared with the substrate (Dec and Bollag 2001), whereas polymerization of 3,4-dichloroaniline (propanil metabolite) by soil microorganisms results in the formation of carcinogenic tetrachloroazobenzene (Pothuluri et al. 1991) (Figure 2). Generally, polymerization products are considered to be unextractable humic components (Dec and Bollag 2000).

Oxidative Nitroaromatic Transformations

In microorganisms as opposed to plants, numerous enzymes from many different pathways are capable of oxidizing nitroaromatic compounds (Table 3), and in many cases the enzymes have been purified, and the genes cloned and sequenced (see review by Zablotowicz et al. 2001). Oxidative reactions that transform various nitroaromatic compounds from several genera of aerobic bacteria have been described (Kadiyala and Spain 1998; Leung et al. 1997; Zablotowicz et al. 1999). In bacteria, monooxygenases, flavin monooxygenases, and dioxygenases are generally involved in the initial oxidation of nitroaromatic pesticides, e.g., 2,4-dinitrophenol can be metabolized by these three enzymes (Cassidy et al. 1999) (Figure 3). Depending on the com-

TABLE 2. Three phases of pesticide metabolism, with pesticide examples and nonspecific chemical reactions.

Phase	Reaction	Example pesticide	Non-specific example of the chemical scheme
I	Oxidation		pesticide + O_2 ⟶ pesticide-O + H_2O
	aryl/alkyl hydroxylation	chlortoluron	
	O-dealkylation	ethametsulfuron	
	N-dealkylation	ethametsulfuron	
	oxidative deamination	metribuzin	
	sulfoxidation	prometryne	
	nitrogen oxidation	credazine	
	Reduction		
	nitroreduction	trifluralin	
	Hydrolysis		pesticide + H_2O ⟶ pest-OH + H-icide
	ester	diclofop-methyl	
	amide	propanil	
	nitrile	cyanazine	
II	Conjugation		pesticide + molecule to conjugate ⟶ pesticide-conjugate
	glucose		
	O-glucoside	metribuzin	
	N-glucoside	flumetsulam	
	glucose ester	2,4-D	
	amino acid	2,4-D	
	glutathione	atrazine	
III	Secondary conjugation		pesticide-conjugate + molecule to conjugate ⟶ pesticide-conjugate-conjugate
	glucose	picloram-glucose	
	malonate	metribuzin-glucose	

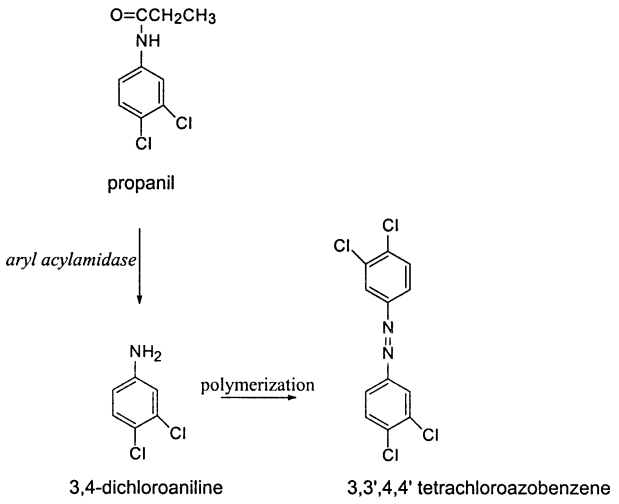


FIGURE 2. Amide hydrolysis of propanil by aryl acylamidase in plants and microorganisms and polymerization of 3,4-dichloroaniline to 3,3′,4,4′ tetrachloroazobenzene by microbial peroxidases.

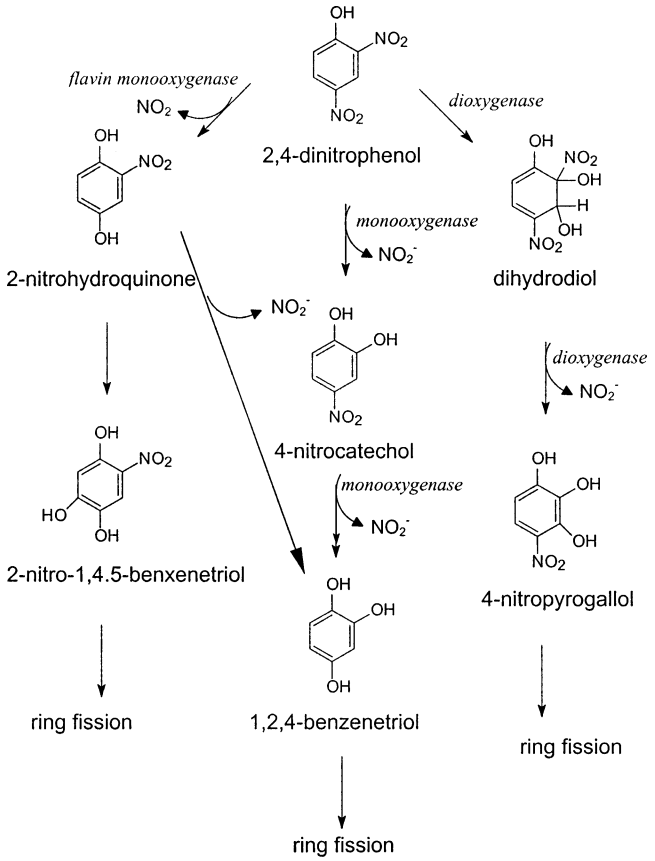


FIGURE 3. Biotransformation of 2,4-dinitrophenol by dioxygenase, *O*-nitrophenol-monooxygenase, or pentachlorophenol-flavin monooxygenase characterized in *Spingomonas* sp. UG30 (adapted from Cassidy et al. 1999, copyright Stockton Press).

pound, nitrite can be released before, or after ring cleavage. The flavin monooxygenase from *Sphingomonas* strain UG30 is responsible for initial nitrite removal from the herbicide 4,6-dinitrocresol, but not dinoseb (4,6-dinitro-*o*-*sec*-butylphenol), because of the steric hindrance caused by the bulky butyl group of dinoseb (Zablotowicz et al. 1999). Overall, these diverse microbial nitroaromatic degradative pathways allow for ring hydroxylation, ring cleavage, and subsequent mineralization of several xenobiotics (e.g., nitrobenzene, nitrobenzoic acid, nitrophenols, and nitrotoluene) (Zablotowicz et al. 2001).

Hydrolytic Transformations

Hydrolytic enzymes cleave bonds of a substrate by adding H or OH from H_2O to each product. There are many hydrolytic enzymes that are capable of metabolizing a variety of substrates, particularly those containing amide, carbamate, or ester functional groups (Table 2). These enzymes may be compartmentalized or extracellular, and reactions can occur under aerobic or anaerobic conditions. Like most classes of enzymes, hydrolytic enzymes may have broad substrate specificities, thereby allowing degradation of a variety of pesticides.

Pesticide ester hydrolysis in plants and microorganisms has been extensively studied and reviewed (Hoagland and Zablotowicz 2001; Incledon and Hall 1997). Ester hydrolysis is commonly carried out by esterases and to a much lesser extent by lipases and proteases. Microbial and plant esterases have a characteristic GLY-X-SER-X-GLY motif (Brenner 1988). The SER acts as a nucleophile, enabling

TABLE 3. Comparison between plant and microbial pesticide metabolism.

Biotransformation	Plants	Microorganisms
General pesticide metabolism	Detoxification	Mineralization
Oxidation	P-450 mediated	Not generally P-450 mediated Mediated by various oxidoreductases
P-450 oxidation	Microsomal membrane bound	Soluble form, not membrane bound
Hydrolytic transformation	Predominantly via esterases, amidases, aryl acylamidases, and nitrilases	Greater enzyme diversity
C–P bond cleavage	None known	Diverse C–P lyases and hydrolytic enzymes
Aromatic nitro-reductive processes	Nitroreductases GSH[a] conjugation	Nitroreductases No GSH conjugation
Reductive dehalogenation	None known	Halo-respiration
Conjugation	With sugar and amino acids Compartmentalized or sequestered GSH conjugation	With xylose, methyl, or acetyl groups Conjugates formed extracellularly No known GSH conjugation

[a] Abbreviation: GSH, glutathione-*S*-transferase.

ester bond cleavage (Cygler et al. 1995). Often, herbicides such as fenoxaprop-ethyl, diclofop-methyl, and 2,4-DB are esterified to increase absorption and selectivity. In plants, the ester bond is metabolized, forming the acid, which is usually more phytotoxic (Table 2). Depending on the herbicide, de-esterification also can result in immediate herbicide detoxification, as is the case with thifensulfuron-methyl (Brown and Kearney 1991) in certain plant species.

Several authors have shown microbial-based ester hydrolysis of diclofop-methyl in soil (Gaynor 1992) and fenoxaprop-ethyl in soil (Köcher et al. 1982; Smith and Aubin 1990) by mixed bacterial consortia (Gennari et al. 1995), and by pure cultures or cell-free extracts (Hoagland and Zablotowicz 1998; Zablotowicz et al. 2000). Four types of esterases have been characterized in *Pseudomonas fluorescens*, each differing in protein structure, cellular localization, and substrate specificity (Choi et al. 1990). Although many microbial esterases have been cloned and sequenced, few have been tested for pesticide hydrolysis.

Atrazine was traditionally considered to be moderately persistent in soil; however, in the past several years many bacterial strains representing several genera have been isolated that can completely mineralize atrazine (Sadowsky and Wackett 2001). These authors have suggested that a unique operon of genes encoding for *s*-triazine degradation has evolved in areas where this herbicide has been used extensively. The gene regions encoding the first three enzymes of atrazine degradation have been isolated and characterized from *Pseudomonas* sp. strain ADP (Boundy-Mills et al. 1997; de Souza et al. 1995, 1996; Sadowsky et al. 1998). This bacterium mineralizes high concentrations (500 mg L^{-1}) of atrazine under both growth and nongrowth conditions, using the herbicide as the sole nitrogen source (Mandelbaum et al. 1995). The *atzA* gene encodes atrazine chlorohydrolase, which dechlorinates atrazine hydrolytically to the nonphytotoxic metabolite hydroxyatrazine (Figure 4). The next step in the degradation pathway is hydrolytic removal of the aminoethyl group from hydroxyatrazine by the *atzB* gene product, hydroxyatrazine ethyl amidohydrolase. Finally, the *atzC* gene encodes for another amidohydrolase that converts *N*-isopropylammelide to cyanuric acid. Martinez et al. (2001) have recently sequenced the complete catabolic plasmid from strain ADP and have identified three additional genes *atzD, atzE,* and *atzF* encoding for cyanuric acid amidohydrolase, biuret hydrolase, and allophanate hydrolase. Thus the total genetic basis for the complete atrazine metabolism in strain ADP has now been identified. Many soil bacteria have the capability to mineralize cyanuric acid (Cook 1987; Cook and Hutter 1981; Erickson and Lee 1989; Korpraditskul et al. 1993). Five other atrazine-degrading bacteria with *s*-triazine–degrading genes have been identified with > 99% homology to *atzABC* from *Pseudomonas* sp. strain ADP, suggesting that horizontal transfer of atrazine degradation genes may have occurred recently (de Souza et al. 1998a, 1998b). In fact, in *Pseudomonas* sp. strain ADP, the three *atz* genes are on a self-transmissible plasmid pADP-1 (de Souza et al. 1998b). Many lines of evidence suggest the ability of microorganisms to mineralize *s*-triazines developed after the first use of these herbicides in the mid-1950s (Sadowsky and Wackett 2001). In contrast to bacteria, atrazine and other *s*-triazines are metabolized in plants via *N*-dealkylation by cytochrome P450s, hydrolytic

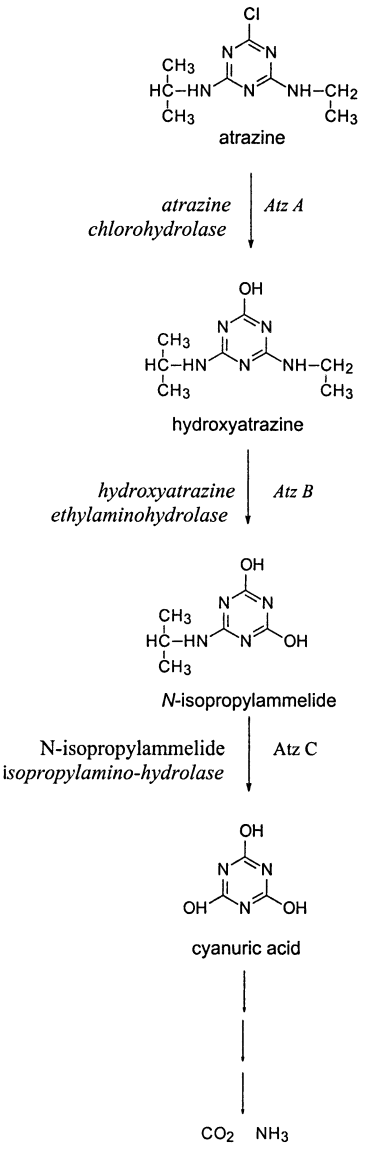


FIGURE 4. Initial degradation pathway of atrazine by *Pseudomonas* sp. stain ADP (Sadowsky and Wackett 2001), with gene designations on the right.

dehalogenation, or displacement of chlorine with glutathione (GSH) (Lamoureux et al. 1998) (Table 2). In microorganisms, there have been no reports of GSH conjugation resulting in dechlorination of *s*-triazines (Zablotowicz et al. 1994).

Propanil is the most widely studied pesticide with regard to amide hydrolysis. Rice (*Oryza sativa* L.) is tolerant to propanil because of high levels of aryl acylamidase, which cleaves the amide bond and is the basis for crop selectivity (Frear and Still 1968) (Figure 2). Aryl acylamidases are widely distributed in plants, bacteria, fungi, and algae (Hoagland and Zablotowicz 2001). After 35 yr of use, mainly for rice production, propanil-resistant barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] has developed and is quite widespread throughout many rice-producing regions of the world (Carey et al. 1995b; Hoagland and Zablotowicz 2001). Propanil resistance is due to enhanced hydrolysis by aryl acylamidase in resistant barnyardgrass (Carey et al. 1995a, 1997) and resistant jungle-rice (*Echinochloa colona*) biotypes (Leah et al. 1994). In several plant species, exper-

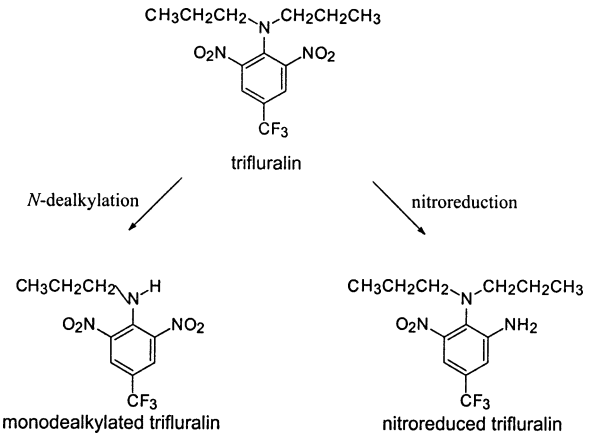


FIGURE 5. *N*-dealkylation of trifluralin observed in peanut (*Arachis hypogaea* L.) and aryl nitroreduction observed in sweet potato (*Ipomoea batatas* L.) and many microorganisms.

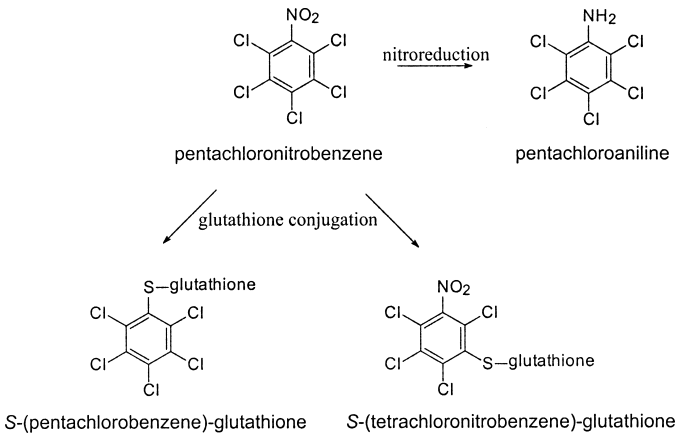


FIGURE 6. Observed metabolism of pentachloronitrobenzene in peanut (*Arachis hypogaea* L.) via aryl nitroreduction, and glutathione *S*-transferase–mediated dechlorination or nitrite release.

iments with propanil analogs (i.e., different ring chloride locations and alkyl chain length, etc.) revealed that generally propanil was the preferred aryl acylamidase substrate (Frear and Still 1968; Hoagland 1975, 1978; Hoagland and Graf 1972). Synergistic interactions can occur when propanil is mixed with any of several agrochemicals (e.g., carbamate and organophosphorus insecticides, and the herbicides anilofos, pendimethalin, and piperophos) (Frear and Still 1968; Matsunaka 1968; Norsworthy et al. 1999). Synergism of propanil and the insecticide carbaryl was the result of competitive inhibition with aryl acylamidases (Bowling and Hudgins 1966; Frear and Still 1968); however, the mechanisms of propanil synergism with other agrochemicals have not been fully characterized. Likewise, certain agrochemicals, e.g., carbamates, can inhibit the hydrolysis of propanil in soil and water by inhibiting microbial aryl acylamidase activity (Kaufman et al. 1971).

The substrate specificity of microbial aryl acylamidases varies even more considerably than that of plant aryl acylamidases. For example, in some *P. fluorescens* strains, the substrate range is limited to the acylanilide pesticides (Hoagland and Zablotowicz 1995), but *Bacillus sphaericus* has a wide substrate range, including acylanilide, phenylcarbamate, and substituted phenylurea pesticides (Engelhardt et al. 1973). Many microbes are capable of amide hydrolysis of propanil (Figure 2). In one study, 37% of 97 bacterial isolates collected from soils and rice flood water of the Mississippi Delta (an area where propanil has been widely used) were capable of hydrolyzing propanil (Hoagland and Zablotowicz 1995). Aryl acylamidases have been purified from several bacterial genera including *B. sphaericus* (Engelhardt et al. 1973), *P. fluorescens* (Hammond et al. 1983), *Pseudomonas pickettii* (Hirase and Matsunaka 1991), *Pseudomonas aeuruginosa* (Riley and Behal 1971), *Nocardia globerula* (Yoshioka et al. 1991), and a coryneform-like bacterium (Mochida et al. 1993). These enzymes range in size from 52.5 to 127 kDa and differ with respect to the subunit aggregation, i.e., some are monomers, dimers, or tetramers. All amidase proteins have a characteristic hydrophobic GLY-GLY-SER-SER motif.

Organophosphorus pesticides are hydrolyzed by microorganisms and have been extensively studied in *Pseudomonas diminuta* (Chaudhry et al. 1988; McDaniel et al. 1988) and *Flavobacterium* spp. (Mulbry and Karns 1989). Hydrolysis, oxidation, and glutathione biotransformations of organophosphorus pesticides appear to be equally important detoxification mechanisms in plants (Lamoureux and Frear 1979). In plants and bacteria, there is limited literature on the role of phosphatases and sulfatases in pesticide metabolism (Hoagland and Zablotowicz 2001). However, there is evidence that sulfatases in the fungi *Trichoderma harzianum* (Katayama and Matsumura 1993) and *P. chrysosporium* (Kullman and Matsumura 1996) hydrolyze the insecticide endosulfan. Nitrile hydrolysis is the main route of metabolism of bromoxynil in wheat (Buckland et al. 1973) and of cyanazine in wheat and potato (*Solanum tuberosum* L.) (Benyon et al. 1972a, 1972b) (Table 2). Hydrolysis of the nitrile group produces an amide moiety that is converted to carboxylic acid, which may be subsequently decarboxylated. In contrast to plants, several bacteria species hydroxylate the cyano group of bromoxynil (Cullimore and Kohout 1974; McBride et al. 1986). Hydrolysis of the carbamate moiety of phenylcarbamate pesticides is common in animals and soil microorganisms but not in plants. In plants, the major metabolic route for the phenylcarbamate pesticides CIPC (Still and Mansager 1972, 1973) and IPC (Dyer and Wright 1959) is aryl hydroxylation and conjugation, rather than hydrolysis of the carbamate moiety (Table 2).

Generally, there is more known about xenobiotic hydrolysis in microorganisms than in plants. However, the precise physiological role of many hydrolytic enzymes is not known. There is a need to further understand the mechanism and regulation of hydrolytic enzymes (Hoagland and Zablotowicz 2001).

Aromatic Nitroreductive Processes

Generally, nitroaromatic compounds are transformed differently in plants in comparison with microorganisms. For example, the major metabolite of trifluralin in peanut (*Arachis hypogaea* L.) is *N*-depropylated trifluralin, whereas in sweet potato (*Ipomoea batatas* L.), the monoamino-derivative of trifluralin is predominant (Probst and Tepe 1969) (Figure 5). In contrast, trifluralin is transformed via nitroreductase by microbes (Lusby et al. 1980). In plants, glutathione conjugation of pentachloronitrobenzene occurs concomitant with the removal of Cl or NO_2 (Lamoureux

and Rusness 1980; Rusness and Lamoureux 1980) (Figure 6). Although glutathione-mediated displacement of the nitro group of aromatic compounds has been described in plants, it has not been reported in microorganisms.

In bacteria, three pathways of reductive metabolism of nitroaromatics have been characterized: aromatic nitroreduction, partial nitroreduction, and hydrogenation (Zablotowicz et al. 2001). Reductive metabolism of nitroaromatic xenobiotics is mediated by nitroreductase enzymes found in aerobic and anaerobic bacteria, and several genera of fungi (Zablotowicz et al. 2001). Nitroreductases are flavoproteins that use NAD(P)H as reducing equivalents, require FMN/FAD as cofactors, and have varying sensitivities to O_2 concentrations. Some bacteria contain multiple aromatic nitroreductase isozymes (Bryant et al. 1981; Kinouchi and Ohnishi 1983). It is sometimes difficult to separate biological and chemical xenobiotic reductions because reduction of aromatic nitrogroups, e.g., trifluralin and diphenyl ether herbicides, may be coupled with anaerobic reduction of humic acids or iron reduction (Oyamada and Kuwatsuka 1989; Probst and Tepe 1969). The conversion of the herbicide acifluorfen to aminoacifluorfen is a common example of an aromatic nitroreduction reaction (Table 2) catalyzed by bacteria under aerobic (Andreoni et al. 1994) and anaerobic (Gennari et al. 1994) conditions, as well as in *Enterobacter cloacae* and *P. fluorescens* cell-free extracts (Zablotowicz et al. 1997). Aminoacifluorfen is susceptible to sorption and incorporation into soil humic material (Locke et al. 1997; Zablotowicz et al. 1997). There is potential to develop transgenic crops that express a bacterial nitroreductase gene to metabolize diphenyl ether herbicides, thereby providing crop tolerance to these herbicides (Zablotowicz et al. 2001).

Numerous bacteria are capable of partial nitroreduction, resulting in NH_3 release and subsequent ring cleavage. Partial nitroreduction pathways are catalyzed by a nitroreductase that reduces the nitro moiety to a hydroxylamino group, followed by further molecular rearrangement catalyzed by a hydroxylaminolyase, forming the hydroxyl amino derivative. Although bacterial partial nitroreduction of several xenobiotics including *p*-nitrobenzoate (Groenewegen and de Bont 1992; Groenewegen et al. 1992) and nitrobenzene (Nishino and Spain 1993) has been demonstrated, partial nitroreduction of pesticides has not been reported. In bacterial partial reductive hydrogenation reactions, the nitroaromatic compound is used as the sole carbon or nitrogen source (Lenke and Knackmuss 1992; Lenke et al. 1992).

Carbon–Phosphorus Bond Cleavage Reactions

Organophosphonates used as pesticides, antibiotics, lubricants, and flame retardants have a carbon-to-phosphorus (C–P) bond, which does not undergo photochemical, hydrolytic, thermal, or chemical degradation (Freedman and Doak 1957). However, many organophosphonate compounds do not persist in the environment because of microbial degradation. Currently, it is believed that plants do not possess the ability to break the C–P bond of organophosphonates, and relatively little is known about fungal organophosphonate metabolism (Bujacz et al. 1995; Sobera et al. 1997; Zboinska et al. 1992). However, degradation of C–P bonds has been extensively studied in bacteria. For instance, a gene cluster designated *phn,* consisting of 17 genes from *Escherichia coli*, is responsible for the degradation of a wide range of phosphonates and is likely to encode for a C–P lyase (Chen et al. 1990; Kim et al. 1993; Metcalf and Wanner 1991; Wackett et al. 1987; Wanner and Boline 1990; Wanner and McSharry 1982; Wanner and Metcalf 1992). The enzyme(s) responsible for direct cleavage of organophosphonate C–P bonds is known by the general name C–P lyase. The ability of C–P lyase to degrade a wide variety of chemically diverse phosphonates is quite striking (Kafarski et al. 2001). However, the precise mechanism of C–P lyase is not fully understood. It is hypothesized that alkanephosphonate biodegradation occurs by two different pathways in which either organophosphonyl (Avila and Frost 1988; Cordeiro et al. 1986; Frost et al. 1987) or organophosphoranyl (Avila and Frost 1989; Wanner and Boline 1990) radicals are formed.

With regard to herbicides, the two-carbon phosphorus bond (C–P–C) of glufosinate is difficult to cleave, and although glufosinate is metabolized in soils, it is not known if the C–P–C bond is broken (Tebbe and Reber 1988). In contrast, many researchers have reported the microbial mineralization of glyphosate in the environment (Cheah et al. 1998; Krzysko-Lupicka and Orlik 1997; Malik et al. 1989; Nomura and Hilton 1977; Ruepple et al. 1977; Sprankle et al. 1975; Zaranyika and Nyandoro 1993) by gram-negative and gram-positive bacteria (Quinn et al. 1989; Ternan et al. 1998), under both anaerobic and aerobic conditions and with no lag phase of degradation (Cheah et al. 1998; Sprankle et al. 1975; Torstensson and Aamisepp 1977). Numerous bacterial strains can use glyphosate as the sole P source without mineralizing it. However, only an *Achromobacter* strain and a *Streptomyces* sp. were able to use glyphosate as the sole carbon or nitrogen source via C–P bond cleavage and formation of sarcosine constitutively in pure culture (Barry et al. 1992; Obojska et al. 1999) (Figure 7). This research indicates that a consortium of microbial species may be required for glyphosate mineralization or that glyphosate is metabolized by fastidious bacteria (Forlani et al. 1999; Kafarski et al. 2001). Two main pathways of glyphosate C–P bond cleavage have been characterized (Ghisalba et al. 1987; Hallas et al. 1988; Quinn et al. 1989; Ternan et al. 1998) (Figure 7); however, neither reaction has been solely used for generating commercially viable genetically engineered glyphosate-tolerant crops. In one pathway, initial cleavage of the C–P bond yields inorganic phosphorus and sarcosine, and the latter is further converted to glycine and a C_1-unit. In the second case, glyphosate oxidoreductase (GOX), a well-characterized 46.1-kDa flavoprotein, cleaves glyphosate into glyoxylate and aminomethylphosphonic acid. Aminomethylphosphonic acid is further degraded by a C–P lyase.

Pesticide Conjugation Reactions

Carbohydrate and Amino Acid Conjugation

Hall et al. (2001b) recently defined pesticide conjugation as the "metabolic process whereby an exogenous or endogenous natural compound is joined to a pesticide or its metabolite(s) facilitating detoxification, compartmentalization, sequestration, and/or mineralization." Conjugation of pesticides often involves utilization of existing enzymatic machinery and is therefore called a cometabolic process. Glucose conjugation to pesticides occurs primarily in plants,

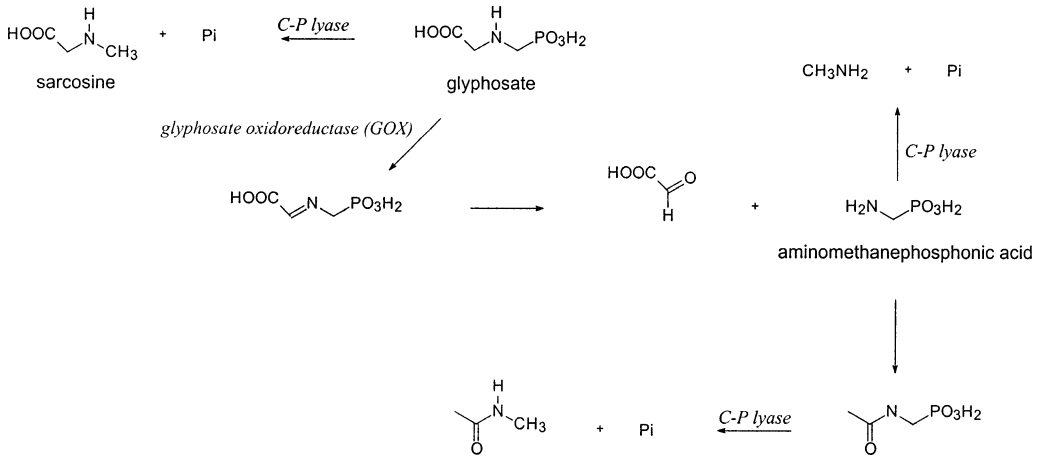


FIGURE 7. Biotransformation of glyphosate, highlighting C–P lyase and glyphosate oxidoreductase (GOX) enzymatic reactions.

resulting in several metabolites including *O*-, *S*-, and *N*-glucosides, glucose ester, gentibioside (e.g., 6-*O*-β-D-glucopyranosyl-D-glucose), and malonyl-glucose conjugates (Table 2). The most common glucose conjugates are *O*-glucosides because pesticide oxidation reactions form hydoxyl groups, which are suitable sites for glucose conjugation.

Differential conjugation of 2,4-D imparts differences of susceptibility in wheat and some broadleaf species. Many susceptible broadleaf weeds produce glucose ester metabolites, which are readily susceptible to hydrolysis, yielding phytotoxic 2,4-D. Conversely, 2,4-D–tolerant wheat rapidly produces amino acid conjugates and *O*-glucosides (Table 2), which are stable nonphytotoxic metabolites that are not easily hydrolyzed. Amino acid conjugation occurs primarily in plants and is very common. Most of the research on amino acid conjugation of pesticides has been conducted on 2,4-D. Twenty amino acids have been found to conjugate with 2,4-D (Andreae and Good 1957; Feung et al. 1971, 1974, 1975).

Uridine diphosphate–glucosyl (UDPG) transferase, an enzyme involved in cellulose biosynthesis, mediates pesticide–glucose conjugation (Klambt 1961) and pesticide–glucose ester conjugation reactions (Mine et al. 1975). As mentioned above, glucose esters of pesticides are cleaved by esterases, often resulting in the release of the pesticide (Frear et al. 1978). However, the addition of a second glucose molecule to the glucose ester produces a gentiobiose conjugate (Hodgson et al. 1973), which is not readily hydrolyzed. Other complex sugar conjugates in addition to gentibioside (two glucose molecules) are glycosides (a glucose and one other sugar, such as arabinose) (Frear 1976).

Pesticide–sugar conjugates can undergo further conjugation with malonate via reaction with malonyl CoA (Sandermann et al. 1997), a common reaction in higher plants (Lamoureux and Rusness 1986). In tomato (*Lycopersicon esculentum* L.), the herbicide metribuzin is conjugated to glucose, which is subsequently conjugated to malonate, forming the *N*-malonyl–glucose conjugate (Frear et al. 1985). A range of UDPG transferase activity within various tomato cultivars confers differential tolerance of these cultivars to metribuzin (Smith et al. 1989). Furthermore, increased metribuzin phytotoxicity in all the cultivars was noted under low light conditions (da Silva and Warren 1976). It was speculated that under low light conditions less glucose and UDPG were produced, thereby reducing conjugation and elevating herbicide phytotoxicity (Frear et al. 1983).

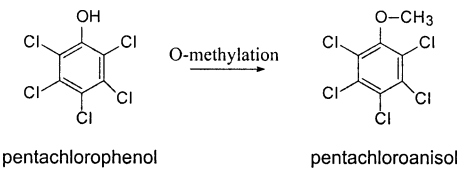


FIGURE 8. Conjugation of pentachlorophenol by microorganisms.

Microbial Pesticide Conjugation

Microbial pesticide conjugation reactions include xylosylation, alkylation, acylation, and nitrosation and can occur intra- or extracellularly. During fungal degradation of lignin, carbohydrates are generated, but toxic phenols are also concomitantly released. These phenols are extracellularly conjugated to xylose as a detoxification mechanism. Fungi use the same process to extracellularly conjugate 2,4-D and 2,4,5-T with xylose (Reddy et al. 1993).

Fungi generally biotransform pesticides and other organic xenobiotics by inducing minor structural changes to the pesticide, rendering it nontoxic (Bollag 1972; Cerniglia 1992). The biotransformed pesticide is released into the soil, where it is susceptible to further metabolism by bacteria. Both fungi and bacteria use methylation as a conjugation reaction to detoxify xenobiotics. For example, formation of *O*-methylated pentachlorophenol by fungal cultures of *Trichoderma virgatum* (Iwan 1976; Joshi and Gold 1993) and many gram-positive and gram-negative bacteria (Häggblom 1990; Suzuki 1983) results in a less toxic, but more recalcitrant, pentachloroanisole (Figure 8). *Phanerochaete chrysosporium* methylates chlorophenoxyacetic acid via a manganese–lignin peroxidase, which is an extracellular degrading enzyme system (Joshi and Gold 1993; Lamar and Dietrich 1990; Valli and Gold 1991).

Pesticide conjugation via microbial acylation with acetate or formate is also common. Phenols and anilines in soil, which are typical breakdown products of phenylacylanilides, phenylcarbamates, and substituted phenylurea pesticides, are often acylated by fungi. For example, the herbicide metobromuron is metabolized by microbes to 4-bromoaniline,

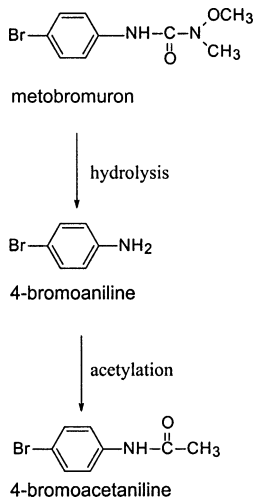


FIGURE 9. Biotransformation of metobromuron by microorganisms.

which is metabolized to 4-bromoacetanilide (Tweedy et al. 1970) (Figure 9).

Nitrosation is a process mediated by bacteria, wherein nitrite reacts with a secondary amine to form a nitrosamine derivative (Alexander 1999; Suzuki 1983). Nitrosamines can be generated by both enzymatic and nonenzymatic processes (Suzuki 1983). Certain pesticides are converted to secondary amines, e.g., dimethyl and dimethylamines, when metabolized in soil (Tate and Alexander 1974).

Plant Glutathione Conjugation Reactions

Glutathione (γ-L-glutamyl-L-cysteinylglycine [GSH]), commonly present in the reduced form, is ubiquitously distributed in most aerobic organisms. Homoglutathione (γ-L-glutamyl-L-cystein-β-alanine), a GSH analog, occurs in several legume species (Macnicol 1987). Although GSH concentrations vary during plant development (Hausladen and Alscher 1993; Rennenberg 1982; Rennenberg and Brunold 1994), GSH is found in relatively high concentrations in most plant tissues (Rennenberg 1982). Glutathione is phloem mobile (De Kok et al. 1986) and is degraded by carboxypeptidases and transpeptidases in the cytoplasm and vacuoles (Steinkamp and Rennenberg 1985). Generally, GSH synthesis is limited by availability of cysteine and hence by the concentration of sulfate ions.

Nonenzymatic GSH conjugation may be important for the metabolism of several herbicides (Rozman and Klaassen 1996). For example, increased GSH concentrations protected wheat from fenoxaprop injury (Romano et al. 1993; Tal et al. 1995). This reaction was considered nonenzymatic because glutathione *S*-transferase (GST) activity in these plants was low (Tal et al. 1995). However, enzymatic conjugation of xenobiotics with GSH via GSTs is more common than nonenzymatic conjugation.

Glutathione-*S*-transferases are homo- or heterodimer, multifunctional enzymes located in the cytosol, which catalyze the nucleophilic attack of the sulfur atom of GSH by the electrophilic center of the substrate (Armstrong 1994; Marrs 1996; Rushmore and Pickett 1993; Tsuchida and Sato 1992). More than 50 plant GST gene sequences from 13 plant species have been published (Dixon et al. 1998a, 1998b; Droog 1997; Marrs 1996; Wu et al. 1999). Compared with other plant and bacterial species, corn (*Zea mays* L.) GST gene enzyme systems have been the most extensively studied (Cole et al. 1997; Frova et al. 1997; Marrs 1996; Sommer and Böger 1999; Timmerman 1989). X-ray crystallography revealed that the N-terminus of this dimeric enzyme is highly conserved and binds GSH at the G-site (Neuefeind et al. 1997a, 1997b; Reinemer et al. 1996; Zajc et al. 1999). The less conserved C-terminal is an α-helix that binds substrates, including herbicides, at the H-site (Neuefeind et al. 1997a, 1997b; Reinemer et al. 1996; Zajc et al. 1999). These two binding domains are kinetically independent (Marrs 1996; Zajc et al. 1999). Recently, a new phylogenetic plant GST classification system was proposed by Dixon et al. (1997, 1998a, 1998b) and Droog (1997) that consists of four classes (I to IV).

In plants and animals, regulation mechanisms and the catalytic function of GST enzymes have been highly conserved during evolution (McGonigle et al. 1997). Some GSTs are constitutively expressed in certain tissues, but GST regulation can be modified by agrochemicals, including herbicide safeners and synergists. It is hypothesized that plant GST gene promoters have multiple regulatory elements that respond differently to specific or more general stress-related signals (Droog 1997). Class I corn GSTs have safener responsive elements, designated by an ATTTCAAA nucleotide sequence (Jepson et al. 1999). Moreover, GSTs probably have common mechanisms of signal transduction to activate gene expression; e.g., all active oxygen species may affect a common transduction pathway during oxidative stress (Low and Merida 1996; Tenhaken et al. 1995).

The role of GSTs and GSH in plants encompasses several major functions. The first is the metabolism of secondary products, including cinnamic acid (Edwards and Dixon 1991) and anthocyanins (Marrs et al. 1995). A second function is regulation and transport of both endogenous and exogenous compounds, which are often GS-X tagged for compartmentalization in the vacuole or cell wall (Hatzios 2001). This is a particularly important aspect for herbicides (Marrs 1996), anthocyanins (Marrs et al. 1995), and indole-3-acetic acid (Bilang and Sturm 1995; Jones 1994). Protection against oxidative stress from herbicides, air pollutants (Sharma and Davis 1994), pathogen attack (Dudler et al. 1991; Taylor et al. 1990), and heavy metal exposure (Hagen et al. 1988; Kusaba et al. 1996) is a third function. Glutathione conjugates and their terminal metabolites are stored in the vacuole or bound to the cell wall (Blake-Kalff et al. 1997; Schröder 1997). Glutathione conjugate pumps in the tonoplast membrane carry GSH conjugates across the membrane (Gaillard et al. 1994; Li et al. 1995a, 1995b; Marrs 1996; Martinoia et al. 1993). In the vacuole, peptidases release the glutathionyl moiety (Schröder 1997).

Glutathione-*S*-transferases in plants were first studied because of their ability to detoxify herbicides (Lamoureux et al. 1991; Marrs 1996). Glutathione-*S*-transferase–based herbicide metabolism imparts herbicide selectivity in several plant species (Cole et al. 1997; Lamoureux and Rusness 1989, 1993; Lamoureux et al. 1991; Marrs 1996; Timmerman 1989; Zajc et al. 1999). Many herbicide families, including sulfonylureas, aryloxyphenoxypropionates, triazinone sulfoxides, and thiocarbamates, are susceptible to GSH conjugation (Cole et al. 1997). Furthermore, there is a positive correlation of both GSH levels and the activity of spe-

cific GST enzymes with the rate of herbicide conjugation and detoxification (Breaux 1987; Breaux et al. 1987; Farago et al. 1993). For example, the resistance of a velvetleaf (*Abutilon theophrasti* Medicus) biotype to atrazine was the result of an enhanced rate of GSH conjugation (Anderson and Gronwald 1991; Gray et al. 1996; Plaisance and Gronwald 1999).

To study GST substrate specificity, Sommer and Böger (2001) purified four recombinant N-terminal 6×His-tagged corn GST isoforms, using an *E. coli* expression system. The recombinant GST isoforms included GST types I, II, III, and IV, with subunits of 29/29, 27/29, 26/26, and 27/27 kDa, respectively. Substrate specificity for each of the four isoforms was different and was based on subunit specificity. For example, GST isoforms with a GST29 subunit could readily conjugate 1-chloro-2,4-dinitrobenzene, whereas isoforms with GST27 subunits had their greatest metabolic activity on thiadiazoliodine and metazachlor. Moreover, the GST27 subunit had metabolic activity on endogenous hydroperoxides such as linolenic acid and cumene hydroperoxide. These results suggest that certain GSTs function through peroxidase activity, to protect the plant from oxidative stress. Based on metabolism levels reported in the literature, the 6×His-tag expression in *E. coli* does not seem to affect GST isoform substrate specificity and is therefore a convenient system to study GST-mediated herbicide metabolism (Sommer and Böger 2001).

Bacterial Glutathione Conjugation Reactions

Compared with plant GSTs, few bacterial GSTs have been characterized at the biochemical level. It is thought that bacterial GST–mediated herbicide metabolism is important because herbicide metabolites with thiol, thioester, and sulfoxide moieties have been identified in soil (Feng 1991; Field and Thurmann 1996). The role of bacterial glutathione conjugation has been demonstrated in the dechlorination of chloroacetamide herbicides, e.g., alachlor (Zablotowicz et al. 1994, 1995) and metolachlor (Hoagland et al., 1997), and the ether bond cleavage of the herbicide fenoxaprop-ethyl (Hoagland and Zablotowicz 1998). Glutathione-*S*-transferases that function as reductive dehalogenases from *Sphingomonas* strains are involved in the dechlorination of pentachlorophenol and lindane (Vuilleumier 2001). In spite of a few well-characterized degradation schemes, little is known about bacterial GST regulation and function (Vuilleumier 2001). Most of the knowledge about bacterial GSTs is based on genomic analysis. Many gene sequences with homology to corn GSTs have been identified within bacterial genomes; however, there is a need to discriminate and determine enzyme function at the biochemical level.

Two messages become clear from bacterial genomic research (Vuilleumier 2001): (1) there is a large set of GST-homologous genes, which vary in size and content in bacteria and (2) certain GST-classified genes are associated with operons and gene clusters involved in xenobiotic dehalogenation. In *E. coli* and *P. aeruginosa* genomes, there are 8 and 17 GST-like genes, respectively (Vuilleumier et al. 1999). In both organisms, only four of the GST-like genes have > 40% homology with known plant and mammalian GSTs at the protein level. The *P. aeruginosa* genes, however, have greater sequence similarity to known biodegradation GST genes, even though the physiological roles of these genes are unknown (Vuilleumier 2001). Nevertheless, known bacterial GSTs have structural similarities to plant and mammalian GSTs despite the extensive variation in sequences (Nishida et al. 1998; Prade et al. 1998; Rossjohn et al. 1998).

The bacterial GST dehalogenases thus far identified include dichloromethane dehalogenase (Cai et al. 1998; Leisinger et al. 1994; Vuilleumier and Leisinger 1996; Vuilleumier et al. 1997), tetrachlorohydroquinone reductase involved in pentachlorophenol metabolism (McCarthy et al. 1996), and 2,5-dichlorohydroquinone reductive dehalogenase involved in lindane degradation (Nagata et al. 1999). An unusual function of a GST enzyme from a *Rhodococcus* strain is the ability to open the epoxide ring during the degradation of isoprene and chlorinated ethenes (van Hylckama et al. 1998). This GST enzyme was purified and the *iso1* gene cloned and characterized (van Hylckama et al. 1999). Similarly, a human GST has been characterized that acts as both an isomerase and a dioxygenase in aromatic ring opening (Tong et al. 1998a, 1998b). This suggests that bacterial GST dehalogenases also may act as isomerases and dioxygenases in aromatic ring opening (Armengaud and Timmis 1997; Fuenmayor et al. 1998; Milcamps and de-Bruijn 1999; Vuilleumier 2001; Werwath et al. 1998). There are many unanswered questions regarding bacterial GST–mediated xenobiotic metabolism, GST regulation, as well as GSH–conjugate uptake, excretion, and toxicity. Moreover, there is potential for using bacterial GSTs in bioremediation and biodetoxification; however, further research is required to fully understand the function and substrate specificity of bacterial GSTs (Vuilleumier 2001).

Formation of Bound Pesticide Residues

Pesticides (mainly conjugated pesticides) are often bound to plant cell walls. Bound pesticide residues are generally considered as those that cannot be extracted with aqueous and organic solvents. However, a more precise definition has been provided by Skidmore et al. (1998):

> "A bound xenobiotic residue is a residue associated with one or more classes of endogenous macromolecules. It cannot be dissociated from the natural macromolecule using exhaustive extraction or digestion without significantly changing the nature of the associated endogenous macromolecules."

When studying bound-pesticide residues using radiolabeled pesticides, it is important to differentiate the bound residue containing the labeled xenobiotic or its metabolite from the "natural label." Natural labeling occurs when $^{14}CO_2$ is released from the mineralized pesticide and is incorporated into the plant cell wall. Natural labeling in plants has been observed with several pesticides (see Sandermann et al. 1983 for review). Furthermore, it is important to know the precise position of the label on the pesticide molecule so that the site of pesticide incorporation into the cell wall can be determined (Sandermann et al. 2001). Digestive treatment with different enzymes such as cellulase, collagenase, pepsin, amylase, and proteases can aid in identifying the nature of pesticide incorporation. On the basis of reports in the literature, it appears that xenobiotics are incorporated randomly into different cell wall components (Sandermann

et al. 2001); however, little is known about the type of linkages involved in this binding.

There is concern about the bioavailability of bound pesticides from plant residues. *Phanerochaete chrysosporium* mineralized bound chloroaniline and 2,4-dichlorophenol, indicating that these compounds may become bioavailable (Arjmand and Sandermann 1985). The ability of animals to release xenobiotics bound to plant residues is unknown. Experiments using a "simulated stomach" demonstrated that pesticides were released from plant residues, but only when high concentrations of bound pesticide residues were used (Sandermann et al. 1990). In comparison, only low concentrations of bound pesticide residues are typically present in plant residues (Sandermann et al. 2001). However, the biological relevance of typically low concentrations of bound pesticide residues is not known. Presently, the U.S. Environmental Protection Agency requires no characterization of bound pesticide residues if concentrations are less than 0.05 ppm of the parent equivalents or 10% of the total pesticide residue. If concentrations exceed these levels, determination of the bioavailability based on "simulated stomach" experiments is required. The toxicological nature and bioavailability of bound xenobiotic residues requires continued research to fully assess its impact on human health and the environment (Sandermann et al. 2001).

Bioremediation and Pesticide Metabolism in the Rhizosphere

The ligninolytic fungus *P. chrysosporium* oxidizes the insecticide lindane by a putative cytochrome P450 enzyme (Mougin et al. 1996, 1997). There is potential to exploit *P. chrysosporium* along with other indigenous microflora to mineralize lindane or convert it to volatile metabolites. Bioremediation of lindane-contaminated soil with *P. chrysosporium* is possible. The advantages of using filamentous fungi for bioremediation include the following: (1) fungi are in direct contact with solid, liquid, and vapor portions of the soil, (2) fungi are capable of transforming a large number of structurally dissimilar compounds, (3) fungi are able to withstand toxic effects of many xenobiotics, and (4) fungi release metabolites, making the metabolites available for further degradation by other microorganisms (Mougin et al. 2001).

Use of Enzymes in Bioremediation

Bioremediation is the use of microorganisms, plants (often called phytoremediation), or biologically active agents to degrade, sequester, or conjugate environmental pollutants. Advantages of bioremediation include ease and timing of application, ability to target specific pollutants, decreased sludge volume, and decreased ecological hazard. There is potential to use enzymatic treatment in bioremediation, and this technology is currently at the laboratory stage of development (Alexander 1999). Advantages of enzymatic treatment over microbial bioremediation include (1) no acclimation phase, (2) use over a wider range of environmental conditions (pH, moisture, temperature), (3) effectiveness at high and low pollutant concentrations, (4) movement of enzymes readily into soil micropores and their protection from inactivation, and (5) little effect of inhibitors of microbial metabolism on enzymes (Dec and Bollag 2001; Nannipieri and Bollog 1991). The disadvantages of enzymatic treatment in bioremediation include the high cost of isolation and storage, the difficulty in maintaining enzyme stability, the requirement for expensive cofactors, and the lack of xenobiotic mineralization (Dec and Bollag 2001).

The use of isolated enzymes to metabolize pesticides is not new (Engelhardt et al. 1973; Kearney and Kaufman 1965; Mulbry and Karns 1989). For example, enzymes from crude *Pseudomonas* cell extracts immobilized on glass beads, hydrolyzed 95% of parathion (10 to 250 ppm) from wastewater (Barik and Munnecke 1982). The same enzyme preparation hydrolyzed parathion at 2,500 ppm in soil and was also effective in hydrolyzing other organophosphate insecticides (triazophos, diazinon, and fenitrothion) (Munnecke 1976). Microbial enzymes with potential for pesticide metabolism include oxidoreductases, hydroxylases, amidases, and esterases. However, enzymatic treatments are not ideal for complete xenobiotic mineralization because mineralization usually requires many enzymes and several cofactors such as NAD(P)H and FAD. Oxidoreductases, such as laccase, tyrosinase, and horseradish peroxidase, can be used to decontaminate soil and water. These enzymes oxidize the substrate to free radicals, which are susceptible to chemical coupling, forming oligomers (Suflita et al. 1981). For example, oligomer formation reactions can take place between humic acid and xenobiotics, resulting in the polymerization of the substrate to soil, as was observed with 2,4-dichlorophenol (Sarkar et al. 1988). In another experiment, horseradish root tissue and hydrogen peroxide (an electron acceptor) decontaminated water containing 850 ppm of 2,4-dichlorophenol and other chlorinated phenols (Dec and Bollag 1994). Depending on the concentration of hydrogen peroxide, up to 100% of the contaminants were removed by polymerization. Furthermore, horseradish root tissue contributed to the irreversible binding of 2,4-dichlorophenol to soil (Flanders et al. 1999).

For enzymatic treatment to be effective in bioremediation, the enzymes must be stabilized. The most effective way to stabilize enzymes is by immobilization. Immobilization can be accomplished by enzyme linkage to organic or inorganic solid supports by adsorption on solid surfaces such as glass, entrapment in polymeric gels, encapsulation, or intermolecular cross-linking (Bickerstaff 1997). Although preparing supports can be time-consuming and expensive, the support can generally be reused. Enzymatic treatment holds great promise in bioremediation of contaminated soil and water.

Pesticide Degradation in the Rhizosphere

Chemicals released by plants may enhance xenobiotic degradation, and it may therefore be beneficial to use plants in the remediation of contaminated soils (Crowley et al. 2001). There are three general mechanisms by which the rhizosphere may act to enhance cometabolism of anthropogenic contaminants (Crowley et al. 2001). First, the rhizosphere may allow selective enrichment of degrader organisms that have densities too low to significantly degrade xenobiotics in root-free soil (Crowley et al. 1997; Jordahl et al. 1997; Nichols et al. 1997). Second, the rhizosphere may enhance growth-linked metabolism or stimulate microbial growth by providing a natural substrate when the concentration of xenobiotics is low or unavailable (Alexander 1999;

Haby and Crowley 1996). Finally, the rhizosphere is rich in natural compounds that may induce cometabolism of xenobiotics in certain microorganisms that carry degradative genes or plasmids. This may permit initial degradation of xenobiotics that would otherwise be unavailable as carbon sources.

Rhizosphere effects on xenobiotic biotransformation have been studied for a variety of compounds, although the mechanisms by which certain plants enhance biodegradation are still poorly understood (Crowley et al. 1997). Differences in plant tolerance to phytotoxic compounds in soils may be related to the plants' ability to induce microorganisms that will detoxify these xenobiotics in the soil environment (Crowley et al. 2001). Research on phytoremediation, through trial and error, has focused on densely rooted, fast-growing grasses and plants, such as *Brassica* sp., with fine root systems. Mulberry (*Morus alba* L.) and poplar (*Populus deltoides*) trees have been used successfully in the phytoremediation of chlorophenols and chlorinated solvents such as trichloroethylene (TCE) (Stomp et al. 1993).

Salicylic acid, flavonoids, and monoterpenes are structurally analogous to many anthropogenic compounds in that they are small, mobile chemicals that are amenable to cellular uptake and may interact through signal transduction pathways to induce the production of specific degradative enzymes (Crowley et al. 2001). For example, salicylic acid was used in tomato fields in irrigation water to promote rhizosphere bacterial growth (Colbert et al. 1993). Salicylic acid is an effective inducer of many different enzymes that may be involved in the cometabolism of xenobiotics such as polyaromatic hydrocarbons (PAHs) and PCBs (Crowley et al. 2001). Degradation of PAHs and PCBs probably evolved in a modular fashion by gene operon recruitment (Williams and Sayers 1994). The salicylic acid–inducible toluene monooxygenase gene, *TOM,* was isolated from *Burkholderia cepacia* (Shields et al. 1995) and introduced into *P. fluorescens* (Yee et al. 1998). The rhizosphere of wheat was inoculated with this transformed *P. fluorescens*, resulting in enhanced degradation of TCE (Yee et al. 1998).

Pseudomonas putida G786 hydroxylates the terpenoid camphor (Bradshaw et al. 1959) by a monoxygenase $P450_{CAM}$ located on a plasmid (Rheinwald et al. 1973). Other P450 enzymes have been implicated in terpene degradation; however, it appears that terpene-induced P450s do not have broad substrate specificity (Crowley et al. 2001). Plants have been used in the phytoremediation of PAH-contaminated soils (Reilley et al. 1996; Schwab et al. 1995), suggesting the involvement of rhizosphere microorganisms in PAH degradation (Trower et al. 1988). A microbial community–based approach may be useful for screening different plant chemicals to find inducers of xenobiotic-degrading enzymes (Crowley et al. 2001).

Reductive Dehalogenation in the Rhizosphere

Reductive dehalogenation is the only significant mechanism for the breakdown of halogenated aromatic, aliphatic, and heterocyclic compounds like PCBs, TCE, hexachlorobenzene, and halogenated pesticides such as heptachlor and aldrin (Barkovskii 2001). Reductive dehalogenation enzymes have broad substrate specificities. There are two principal mechanisms of RDE. The first process is cometabolic RDE, which yields no energy for the organism. The second mechanism is halorespiration, where organohalides act as terminal electron acceptors and adenosine triphosphate is generated (Griffith et al. 1992). In cometabolic RDE, organohalides are not used as terminal electron acceptors. Generally, anaerobic respiration is relatively inefficient in that electrons produced during substrate oxidation lose energy in the electron transport chain. In cases of excess substrate, high-energy electrons, "hot electrons," accumulate, creating a redox potential imbalance. However, microbes may gain protection from "hot-energy" electrons by halo-scavenging, thereby providing an advantage to microbes that conduct halorespiration (Barkovskii 2001).

In theory, certain microsites within the rhizosphere are favorable (anaerobic conditions, low redox potentials, and available electron acceptors) for RDE thus fascilitating the transformation of halogenated compounds (Barkovskii 2001). Although O_2 does not inhibit RDE (Criddle et al. 1986; Häggblom et al. 1989; Steiert and Crawford 1986; van den Tweel et al. 1987), RDE is generally an anaerobic process. Spatial and temporal heterogeneity in O_2 distribution in the rhizosphere environment usually (but not always) provides microbes with localized environments that are anaerobic and have low redox potential, thereby favoring RDE reactions (Barkovskii 2001). Moreover, most of the terminal electron acceptors, such as nitrate (Haider et al. 1987), ferric iron (Frenzel et al. 1999; Wang and Peverly 1999), sulfate (Blaabjerg and Finster 1998), CO_2 (Frenzel et al. 1999; Roden and Wetzel 1996), and quinones (Barkovskii et al. 1994, 1995), are abundant in the rhizosphere. The addition of quinones can enhance both the capacity and the rate of microbial reduction of contaminants (Barkovskii and Adriaens 1998; Barkovskii et al. 1995; Lovley et al. 1996). In RDE, quinones and semiquinones provide reducing power and protons, which transfer excess electrons to organohalides.

The bioavailability of hydrophobic contaminants determines the rate of xenobiotic transformation and mineralization. For example, the rhizosphere may increase the bioavailability of lipophilic polyhalogenated aryl halide contaminants (Banks et al. 1999; Erickson et al. 1995; Fan et al. 1997; Ferro et al. 1994; Hustler and Marschner 1994; Nardi et al. 1997) otherwise unavailable for RDE. Concomitantly, the bioavailability of the hydrophilic intermediates of organohalide degradation will decrease, thus reducing further degradation (Kreslavski et al. 1999; Walton et al. 1994). Further research is needed to fully characterize the role of the rhizosphere in halide degradation and the biotransformation of xenobiotics.

Herbicide Metabolism and Crop Safeners

Metabolism-Based Crop Tolerance to Herbicides

One of the major mechanisms of herbicide selectivity between crop and weed species is based on differential rates and routes of metabolism. For example, the mechanism of flumetsulam selectivity is based on the rapid rate of flumetsulam hydroxylation and glycosylation in corn compared with the relatively slower rate in lambsquarters (*Chenopodium alba* L.) (Frear et al. 1993). Substitutions at the 5-position of many imidazolinone herbicides dramatically affect their selectivity by altering the rate and route of metabolism in crops such as peanut, corn, and soybean (*Glycine*

max), thus providing selectivity between these crops and their associated weeds (Shaner and Mallipudi 1991; Tecle et al. 1993).

Many crops have been developed through breeding or molecular biology that use enhanced herbicide metabolism as the basis of herbicide tolerance. Classical plant-breeding techniques have been used to develop metribuzin-tolerant soybeans based on enhanced herbicide metabolism (Barrentine et al. 1976, 1982; Hardcastle 1974, 1979; Hartwig et al. 1980; Mangeot et al. 1979). The *tfdA* gene from the bacteria *Alcaligenes eutrophus*, encoding for a 2,4-D–degrading enzyme, was isolated, cloned (Streber et al. 1987), and introduced into tobacco (*Nicotiana tabacum*) (Stalker et al. 1996) and cotton (*Gossypium hirsutum* L.) (Bayley et al. 1992; Llewellyn and Last 1996), imparting 2,4-D tolerance to these crops (crops not commercialized). Bromoxynil is used as a nitrogen source by *Klebsiella pneumoniae* subsp. *ozaenae* (McBride et al. 1986). The bromoxynil-nitrilase (*bxn*) gene from this organism has been cloned and the protein characterized (Stalker and McBride 1987; Stalker et al. 1988a). The *bxn* gene was introduced into several crop species, thereby producing bromoxynil-resistant plants through rapid hydroxylation of bromoxynil (Stalker et al. 1988b). Bromoxynil-tolerant canola (*Brassica napus* L.), sugar beet (*Beta vulgaris* L.), and potato (*Solanum tuberosum* L.) are now under development (Shaner and Tecle 2001). Moreover, the tolerance of transgenic crops with resistance to the synthetic herbicide glufosinate and phosphinothricin (the natural product) is based on enhanced metabolism. Two genes for acetyltransferase, *bar* and *pat*, were isolated from *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, respectively. Both genes have been used to produce glufosinate-tolerant crops. Once acetylated, glufosinate does not inhibit glutamine synthetase. For engineering tolerance to glyphosate in crop plants, the *GOX* gene isolated from *E. coli* was fused with the chloroplast transit peptide from the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Barry et al. 1992; Padgette et al. 1996). This construct led to high steady-state *GOX* protein production and resulted in the development of some glyphosate-tolerant crop species. However, glyphosate tolerance in several transgenic crops is due to a herbicide-insensitive target site, namely CP4 5-enolpyruvylshikimate-3-phosphate synthase.

Most plants do not rapidly de-esterify the pyridine herbicide thiazopyr. However, Feng and Ruff (2001) transformed tobacco and tomato plants expressing rabbit liver esterase (*rle3*) that de-esterified thiazopyr; consequently, the plants were resistant to its phytotoxic effects. The *RLE3* enzyme and thiazopyr were chosen because (1) a single detoxification step is involved, (2) only one enzyme is required, (3) the enzyme has favorable kinetics resulting in rapid detoxification, (4) the enzyme is stable and abundant, allowing for purification and cloning with relative ease, and (5) the herbicide metabolite is nontoxic and not prone to reactivation. Thiazopyr tolerance was directly proportional to the extent of *RLE3* esterase expression. After constitutive expression of *RLE3,* a broad-substrate esterase, there were no phenotype differences between transformed and wild-type plants. This may be due to the fact that enzyme localization is in the endoplasmic reticulum or because the enzyme is not active against endogenous substrates. In the future, researchers designing crop–herbicide tolerance should consider capitalizing on temporal expression during germination, when the plant is typically exposed to this herbicide (Feng and Ruff 2001).

Chemical Safeners

The use of chemical agents to protect crops from herbicide injury was first conceptualized in the late 1940s by Hoffman (1962). Also called antidotes, protectants, and antagonists, the name safener is preferred (Hatzios and Hoagland 1989) because these chemicals are used to prevent herbicide injury and do not reverse phytotoxic effects. To date, no commercial broadleaf-crop safeners have been developed. Safeners are used mainly in corn, grain sorghum (*Sorghum bicolor* (L.) Moench.), and to a lesser extent in wheat and barley (*Hordeum vulgare* L.), with the following herbicide families: aryloxyphenoxypropionates, sulfonylureas, imidazolinones, chloroacetamides, thiocarbamates, and sulfonamides. There are several chemical classes of safeners, including the chloroacetamides (dichlormid), naphthopyranone derivatives, phenyl triazoles (fenchlorazole-ethyl [FCE]), oxime ether derivatives (cyometrinil), and 2,4-disubstituted-5-thiazolecarboxylates (flurazole).

Three possible modes of safener action include (1) reduced herbicide absorption or translocation (or both), (2) competitive inhibition between the safener and the herbicide at the target site, and (3) enhanced herbicide detoxification. Various safeners have been shown to increase (Ekler et al. 1993; Fuerst and Gronwald 1986; Gronwald et al. 1987; Yenne et al. 1990), decrease (Ezra et al. 1982; Han and Hatzios 1991; Ketchersid et al. 1982; Thiessen 1978), or have no influence on (Davies et al. 1993, 1997) herbicide uptake. In general, the effects of safeners on uptake and translocation do not appear to be a widely important mechanism of safener action (Ramsey et al. 2001). Although there are significant structural similarities between specific safeners and herbicides (Stephenson et al. 1978, 1979; Yenne and Hatzios 1990), safener antagonism of herbicide action at the target site is also an unlikely mechanism of action (Ramsey et al. 2001). Most research indicates that increased herbicide metabolism is the most likely mechanism of safener action (Hatzios and Hoagland 1989).

Safeners enhance one or several of the following metabolic reactions and enzymes: cytochrome P450s (Kreuz et al. 1996; Leavitt and Penner 1979), hydroxylation (Kreuz et al. 1991; Lamoureux and Rusness 1991), hydrolysis (Pallet et al. 1998), glycosylation (Kreuz et al. 1991; Lamoureux and Rusness 1991), carboxyesterases (Hatzios 1997; Yaacoby et al. 1991), and GSH conjugation (Lamoureux and Rusness 1991). The effect of safeners on the regulation of P450s and other oxidative enzymes remains largely unknown (Durst and O'Keefe 1995). Safeners may act to increase GSH content and GST activity (Carringer et al. 1978; Ekler and Stephenson 1989; Ezra and Gressel 1982; Gronwald et al. 1987; Lay and Casida 1976; Timmerman 1989; Zama and Hatzios 1986), and may also interfere with feedback inhibition of GSH synthesis resulting in continuous GSH production (Tal et al. 1993). The mechanism of safener-induced GST activity is not known, but it is likely due to increased de novo GST synthesis (Jepson et al. 1994). Furthermore, little is known about the effect of safeners on the fate of GSH conjugates, but it is generally accepted that safeners do not alter the fate of GSH conjugates (Hatzios

2001). Safeners also influence both the activity of existing membrane transporter proteins (Gaillard et al. 1994) and the expression of genes coding for membrane transporter proteins (Sanchez-Fernandez et al. 1998).

The question remains, "Why don't safeners protect weeds from herbicide injury?" In cases where safeners are used as seed treatments, weeds are not exposed. With foliar-applied safeners, for example, FCE, differences in the rate and route of metabolism between crop and weed species result in selectivity (Stephenson et al. 1993). Generally, crop species such as wheat and barley intrinsically contain more GSH and cysteine than do most weed species (Ekler et al. 1993; Gronwald et al. 1987; Tal et al. 1993). For instance, FCE increased GSH content in wheat and barley but not in the weed crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Yaacoby et al. 1991). Although FCE increases the rate of metabolism of fenoxaprop-ethyl to the active herbicide fenoxaprop in both weed and crop species, fenoxaprop was subject to more rapid GSH conjugation in the crop species than in crabgrass. Therefore, FCE acts as a synergist in crabgrass and as a safener in wheat and barley.

Chloroacetamide safeners affect both P450- and GSH-mediated herbicide detoxification, which suggests that safeners may act on a central stress response system, whereby a cascade of events prepares the plant to reduce oxidative stress (Ramsey et al. 2001). Researchers have used safeners to induce herbicide-metabolizing genes (Barrett 1998) and to study safener-responsive genes (Hershey and Stoner 1991). Future research will likely reveal that there are multiple mechanisms of safener action.

In Vitro Methods for Studying Pesticide Metabolism in Plants and Microorganisms

In vitro systems include cell and tissues cultures, cell extracts, purified enzymes, or subcellular fractions (e.g., microsomes). In vitro systems are very powerful tools to help elucidate microbial, plant, and mammalian pesticide metabolism. In vitro methods (Schmidt 2001; Schocken 2001) allow for the (1) prediction of metabolites that are likely present before initiation of an in vivo study, (2) generation of metabolites in sufficient quantities for identification, (3) detection of intermediate metabolites, which may provide insight into the metabolic pathway, (4) characterization of nonextractable residues, (5) "metabolic profiling" to determine the rate and pattern of metabolism between species, and (6) determination of genetics and enzymology of the metabolic pathway. An advantage of cultures (i.e., microbial, plant, or animal) is that high pesticide concentrations (i.e., concentrations that exceed the pesticide water solubility) can be used because the metabolite produced by the cells shifts the equilibrium, allowing more pesticide to dissolve in the culture medium (Schocken 2001). This procedure allows for the formation of metabolites at very high concentrations, which is valuable for metabolite identification by spectroscopic analysis.

A nonbiological, in vitro method was developed that "mimics" cytochrome P450 oxidation. This method is an ascorbic acid oxidation system that degrades pesticides by *N*-dealkylation or hydroxylation of aromatic rings and methyl groups (Balba and Saha 1974). Thus, there is potential to use this nonbiological method and others such as acid or base hydrolysis to predict the type of metabolites that may be produced by plants, animals, and microorganisms.

Plant cell suspension culture is commonly used to study pesticide metabolism. Many different systems can be used, from heterotropic (i.e., dark-grown cells) to fully phototrophic (autotrophic) systems. Typically, the heterotropic in vitro method is preferred because metabolism is strictly due to plant enzymes and not microorganisms (because of sterile techniques) or photolysis (Schmidt 2001). Furthermore, the lack of chlorophyll makes sample preparation, purification, and metabolite identification less difficult. It is generally accepted that heterotropic cultured plant cells metabolize pesticides in a similar manner as whole plants, but differ in the quantity of metabolites formed because in vitro cultures essentially have no barriers to pesticide penetration or translocation and little or no bound-pesticide residues are formed.

Plant cell suspension cultures are extremely useful for determining metabolic patterns or "metabolic profiling" (Schmidt 2001). Metabolic profiling uses cell cultures from different crop and weed species to obtain a qualitative approximation of pesticide metabolism. Thus, comparisons between species can be correlated with the quantity of pesticide metabolized and the type of metabolites formed. For example, parathion was metabolized by cell suspensions of five plant species to the same metabolites (paraoxon, 4-nitrophenol, and 4-nitrophenol-glucoside) (van der Krol et al. 1995). However, the rate of metabolism was different among species, i.e., the quantity of nontransformed parathion remaining ranged from 5.6 to 75.6%. Although previously questioned, both glufosinate (Komoßa and Sandermann 1992) and glyphosate (Komoßa et al. 1992) were shown to be metabolized by plants using plant cell suspension cultures. In another example, plant cell cultures were used to identify the metabolites of pyrene, which would be severely limited in planta because of the low levels of uptake and translocation (Hückelhoven et al. 1997). In all the aforementioned examples, at least some of the metabolites produced by plant cell suspension cultures also were observed at the whole-plant level or in the soil environment. Although in vitro methods are not intended to replace whole-organism studies, they do provide a relevant, rapid approach to study pesticide metabolism.

Pure fungal or bacterial cultures of genera such as *Streptomyces*, *Bacillus*, *Pseudomonas*, *Cunninghamella*, and *Aspergillus* are typically used to examine xenobiotic metabolism (Schocken 2001). In one study, 41 cultures of common soil fungi and bacteria were screened for their ability to metabolize clomazone (Liu et al. 1996). Seventeen species metabolized clomazone to many different metabolites. Some of these metabolites had been previously found in planta in soybean, but many were difficult to synthesize and identify. In some instances, microbial cultures metabolize xenobiotics in a similar manner as plants and animals (Feng and Wratten 1989; Liu et al. 1996; Schocken et al. 1997). Thus, microbial in vitro systems may be good predictors of the metabolites that will be found in plants, animals, and the environment. Such microbial systems may also be useful to generate metabolites in quantities so that identification may be facilitated.

Conclusions

The basis for selectivity of plants and microorganisms to xenobiotics has been extensively studied during the past 40 yr and has provided a wealth of information on diverse biological processes and enzymes in plants and microorganisms. Understanding the plant enzymatic systems involved in metabolic processes provides a basis for developing novel, more effective, and environmentally benign herbicides and safeners. Microorganisms have similar herbicide-degradative processes and, unlike plants, an intrinsic nature for rapid genetic adaptation to chemicals in the environment. The detoxification and degradation potential of individual microorganisms is being exploited for remediation of soil and water contaminated with pollutants of diverse chemical nature. One particular strategy is phytoremediation, a process by which plants and their associated microorganisms collectively degrade, detoxify, and remove pollutants. Correspondingly, microbial genes that encode for pesticide detoxification–degradation pathways have become pivotal in the production of herbicide-tolerant crops. Many detoxification mechanisms are common to both higher plants and prokaryotic organisms. However, unique mechanisms for xenobiotic transformation are continually being elucidated in plants and microbes while novel xenobiotics and natural products also are being discovered. Biotechnology methods have changed pest control dramatically within the past 10 yr, and exciting new directions are continually being explored. Nevertheless, these advances must still rely on further developments in whole-plant and microbial physiology.

It is intended that this review and the ACS book discussed herein should serve as valuable information sources for those interested in pesticide biotransformation, metabolism, and fate. It is particularly hoped that this review will motivate researchers to become involved in the aforementioned areas of plant and microbial enzyme research. The answer to many current questions may be found in understanding the mechanisms, specificity, stability, regulation, and expression of enzymes involved in pesticide metabolism. Ultimately, it is hoped that this knowledge will promote an understanding of the safe and economical use of pesticides.

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